

DP Barcode: D215429

MRID No.: 432600-03

DATA EVALUATION RECORD
§ 72-3(B) -- ACUTE EC₅₀ TEST WITH AN ESTUARINE/MARINE MOLLUSK
SHELL DEPOSITION STUDY

1. **CHEMICAL:** Didecyldimethylammoniumchloride PC Code No.: 069149
(DDAC)

2. **TEST MATERIAL:** [¹⁴C]DDAC Purity: 84.6%

3. **CITATION**

Author: Emily Dionne
Title: Didecyldimethylammoniumchloride (DDAC):
Evaluation in a Static (Recirculated)
Acute Toxicity Test with Eastern Oysters,
(Crassostrea virginica) Using [¹⁴C]DDAC

Study Completion Date: March 15, 1994

Laboratory: Springborn Laboratories, Inc.
Wareham, MA

Sponsor: Lonza Inc.
Fair Lawn, NJ

Laboratory Report ID: 93-6-4854

MRID No.: 436200-03

DP Barcode: D215429

4. **REVIEWED BY:** Christian M. Newman, Wildlife Biologist,
KBN Engineering and Applied Sciences, Inc.

Signature: *C. M. Newman*

Date: 11/3/85

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,
KBN Engineering and Applied Sciences, Inc.

Signature: *P. Kosalwat*

Date: 11/3/85

5. **APPROVED BY:** ^{HENRY T. CAMERON} ~~Ann Stavola~~, Head of Section ⁴(5), EEB, EFED

Signature: *Henry T. Cameron*

Date: 2-21-96

6. **STUDY PARAMETERS**

Age or Size of Test Organism: 26-39 mm valve length

Definitive Test Duration: 96 hours

Study Method: Static

Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute oyster shell deposition test. Based on mean measured concentrations, a 96-hour EC₅₀ of 0.094 ppm ai classifies DDAC as very highly toxic to oysters. The NOEC is 0.063 ppm ai.

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Results Synopsis

EC₅₀: 0.094 ppm ai

95% C.I.: 0.017-0.21 ppm ai

NOEL: 0.063 ppm ai

Probit Slope: 6.26

8. ADEQUACY OF THE STUDY

A. Classification: Core.

B. Rationale: See the reviewer's comments in Section 15.

C. Repairability: N/A.

9. BACKGROUND

10. GUIDELINE DEVIATIONS

1. The test was conducted using a static system with circulated solutions within the test chambers.

11. SUBMISSION PURPOSE:

12. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species are the Pacific oyster (<i>Crassostrea gigas</i>) and the Eastern oyster (<i>Crassostrea virginica</i>)	<i>Crassostrea virginica</i>
<u>Mean valve height</u> 25-50 mm along the long axis	30 (\pm 3) mm
<u>Supplier</u>	P. Cummins Oyster Co., Pasadena, MD
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Not reported

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 10 days	13 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	N/A
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
<u>Amount of peripheral shell growth removed prior to testing</u>	3-5 mm
<u>Feeding during the acclimation</u> Must be fed to avoid stress.	Algal diet of <i>Isochrysis galbana</i> and <i>Tetraselmis maculata</i>
<u>Pretest Mortality</u> <3% mortality 48 hours prior to testing	<1% mortality was reported in the last seven days prior to test initiation

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Natural unfiltered seawater from an uncontaminated source.	Natural unfiltered seawater from the Cape Cod Canal, Bourne, Massachusetts
Does water support test animals without observable signs of stress?	Yes
<u>Salinity</u> 30-34 ‰ salinity, weekly range <6‰.	31-32 ‰
<u>Water Temperature</u> 15-30° C, consistent in all test vessels	21-22°C

Guideline Criteria	Reported Information
pH	7.2-8.0
Dissolved Oxygen ≥60% of saturation throughout	Range: 76-95% of saturation during the test
Total Organic Carbon	2.0 mg/L
Test Aquaria Should be constructed of glass or stainless steel.	Glass rectangular chambers (49.5 x 25.5 x 29 cm) with 18 liters of dilution water or test solution
Type of Dilution System Must provide reproducible supply of toxicant	Static test with the test solution continuously circulated within each test chamber by an impeller pump
Flow rate Consistent flow rate	The flow rate generated by a pump within each chamber was 5.25 L per oyster per hour
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Yes
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L	N/A

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If EC ₅₀ >100 mg/L with 30 oysters, then no definitive test is required.	After 96 hours, 100%, 100%, and 80% mortality occurred at 0.9, 3.0, and 0.27 ppm, respectively. Twenty percent reduction in growth occurred at 0.081 ppm and no reduction at 0.024 ppm.

Guideline Criteria	Reported Information
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	A control and 0.027, 0.047, 0.076, 0.13, and 0.21 ppm ai.
<u>Number of Test Organisms</u> Minimum 20 individual per test level and in each control	20 oysters
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured hourly in at least one chamber 2. <u>DO and pH</u> Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	1) Temperature measured hourly in the dilution water control. 2) DO and pH measured daily in each chamber.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes

13. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes

Guideline Criteria	Reported Information
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0%
Control Shell Deposition Must be at least 2 mm.	3.1 mm
Recovery of Chemical	Mean recovery ranged from 79 to 89% of nominal
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes, reduced feeding, fecal and pseudofecal production were observed in oysters exposed to the two highest test concentrations.

Shell Growth

Concentration (ppm)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Decrease
Nominal	Mean Measured				
Control	—	20	0	3.1	—
Solvent Control	N/A	—	—	—	—
0.027	0.023	20	0	2.7	12
0.047	0.037	20	0	3.2	0(+3.9)*
0.076	0.063	20	0	2.6	15
0.13	0.11	20	0	1.1	65
0.21	0.19	20	14	0.07	98

* indicates higher shell growth than the control.

B. Statistical Results

Method: Linear Regression (Probit analysis)

96-hr EC₅₀: 0.1 ppm ai

95% C.I.: 0.017-0.21 ppm ai

Probit Slope: 4.43

NOEC: 0.063 ppm ai

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Statistical Method for EC ₅₀	Probit analysis
EC ₅₀ (95% C.I.)	0.094 (0.087-0.100) ppm ai
Probit Slope	6.26
Statistical Method for NOEC	Williams' test
NOEC	0.063 ppm ai

15. REVIEWER'S COMMENTS: This study did not follow the SEP or ASTM procedures for an oyster shell deposition test. The study was conducted using a static method with the test solution continuously circulating within each test chamber. Test concentrations decreased substantially in all test solutions by the time the second measurement was conducted (i.e., at 48 hours) (Table 2, attached). However, the decrease in test concentrations during this 48-hour period was much less than what was observed in the other test submitted (MRID No. 436200-02, DDAC/Oyster Shell Deposition). The results from this study also yielded a lower 96-hour EC₅₀ (i.e., 0.09 ppm ai) which would place DDAC in the "very highly toxic" category. Therefore, this study will be accepted as Core.

Table 2. Concentrations of [^{14}C]DDAC (total residues) measured in exposure solutions during the 96-hour flow-through exposure of Eastern oysters (*Crassostrea virginica*).

Nominal ^a Concentration (µg A.I./L)	Measured Concentrations (µg A.I./L) ^a							Percent Nominal (%)
	0-Hour		48-Hour		96-Hour		Mean (SD) ^b	
	#1	#2	#1	#2	#1	#2		
Control	<0.16	<0.16	<0.16	<0.16	<0.17	<0.16	NA	
27	34	41	19	17	14	14	23 (11)	85
47	58	53	33	29	25	26	37 (14)	79
76	86	86	52	54	50	51	63 (18)	83
130	130	130	93	96	96	93	110 (20)	84
210	230	220	170	170	170	170	190 (32)	89
QC ^c #1	43.5 (45.5) ^d		121 (152)		32.2 (37.9)			
QC #2	68.8 (75.8)		25.2 (30.3)		37.9 (45.5)			
QC #3	136 (152)		37.7 (45.5)		131 (152)			

^a Nominal and measured concentrations were corrected to reflect a radiopurity of 84.6% for the [^{14}C]DDAC.

^b Mean measured concentrations are presented with the standard deviations in parentheses and were calculated using the actual analytical results rather than the rounded (two significant figures) values presented in this table.

^c QC = Quality Control sample.

^d Value in parentheses represents nominal fortified concentration for each QC sample.

KOSALWAT DDAC CRASSOSTREA VIRGINICA 11-02-95

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.19	100	98	98	0
.11	100	65	65	0
.063	100	15	15	0

THE BINOMIAL TEST SHOWS THAT .063 AND .11 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 9.397274E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	1.869462E-02	9.449194E-02	8.792595E-02	.1009724

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	3.390898E-02	1	.5093242

SLOPE = 6.261039
95 PERCENT CONFIDENCE LIMITS = 5.108107 AND 7.413971

LC50 = 9.355458E-02
95 PERCENT CONFIDENCE LIMITS = 8.725778E-02 AND .1000773

LC10 = 5.864397E-02
95 PERCENT CONFIDENCE LIMITS = 5.125406E-02 AND 6.478712E-02

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DDAC(C14)/OYSTER SHELL DEPOSITION TEST

File: h:\project\epa\ddacc14.tox

Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	CONTROL	20	0.900	5.800	3.055
2	0.023	20	0.000	5.800	2.700
3	0.037	20	1.200	4.800	3.175
4	0.063	20	1.000	4.400	2.590
5	0.11	20	0.000	4.100	1.055

DDAC(C14)/OYSTER SHELL DEPOSITION TEST

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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	CONTROL	2.289	1.513	0.338	49.52
2	0.023	2.000	1.414	0.316	52.38
3	0.037	0.851	0.923	0.206	29.06
4	0.063	0.966	0.983	0.220	37.95
5	0.11	1.101	1.049	0.235	99.44

DDAC(C14)/OYSTER SHELL DEPOSITION TEST

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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	57.973	14.493	10.055
Within (Error)	95	136.935	1.441	
Total	99	194.908		

Critical F value = 2.53 (0.05,4,60)

Since $F > \text{Critical } F$ REJECT H_0 : All equal

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	CONTROL	20	3.055	3.055	3.055
2	0.023	20	2.700	2.700	2.938
3	0.037	20	3.175	3.175	2.938
4	0.063	20	2.590	2.590	2.590
5	0.11	20	1.055	1.055	1.055

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
CONTROL	3.055				
0.023	2.938	0.309		1.67	k= 1, v=95
0.037	2.938	0.309		1.75	k= 2, v=95
0.063	2.590	1.225		1.77	k= 3, v=95
0.11	1.055	5.268	*	1.78	k= 4, v=95

s = 1.201

Note: df used for table values are approximate when v > 20.

11/3/95

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DRAFT

MRID No.: 432600-03

436200-03

DATA EVALUATION RECORD
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1. **CHEMICAL:** Didecyldimethylammoniumchloride PC Code No.: 069149 (DDAC)

2. **TEST MATERIAL:** [¹⁴C]DDAC Purity: 84.6%

3. **CITATION**

Author: Emily Dionne
Title: Didecyldimethylammoniumchloride (DDAC): Evaluation in a Static (Recirculated) Acute Toxicity Test with Eastern Oysters, (*Crassostrea virginica*) Using [¹⁴C]DDAC

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4. **REVIEWED BY:** Christian M. Newman, Wildlife Biologist,
 KBN Engineering and Applied Sciences, Inc.

Signature:  **Date:** 11/3/95

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,
 KBN Engineering and Applied Sciences, Inc.

Signature: P. Kosalwat **Date:** 11/3/95

5. **APPROVED BY:** Ann Stavola, Head of Section (5), EEB, EFED

Signature: **Date:**

6. **STUDY PARAMETERS**

Age or Size of Test Organism: 26-39 mm valve length

Definitive Test Duration: 96 hours

Study Method: Static

Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute oyster shell deposition test. Based on mean measured concentrations, a 96-hour EC₅₀ of 0.094 ppm ai classifies DDAC as very highly toxic to oysters. The NOEC is 0.063 ppm ai.

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4	0.063	20	2.590	2.590	2.590
5	0.11	20	1.055	1.055	1.055

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